

FOURTH REPORT OF THE WELLCOME TROPICAL RESEARCH
LABORATORIES, KHARTOUM

The alkalinity of the Blood Serum in Kala Azar

R.G.Archibald.

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KHARTOUM

VOLUME A.—Medical

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THE ALKALINITY OF THE BLOOD SERUM IN KALA-AZAR

BY

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The recent researches of Nierenstein¹ on the acidity and alkalinity of the blood in trypanosomiasis infections suggested the idea that a similar line of researches might be carried out in kala-azar, with the object of ascertaining if any change occurred in the blood serum of patients suffering from this disease.

Owing to the fact that kala-azar is still a somewhat uncommon disease in the Sudan, it was difficult to obtain sufficient material for an extensive number of tests, and although these results were obtained from only four undoubted cases of kala-azar, they are published in the hope that some specific line of treatment may be adopted in accordance with the changes that have been found to exist in the blood serum of patients suffering from this fatal disorder.

The tests were carried out in patients in whom the presence of the Leishman body had been previously demonstrated by the microscope.

The technique employed was that recommended by Moore and Wilson,² and the tests were conducted on the "entire serum" in preference to the whole blood, as Wright³ has pointed out that from a clinical point of view the alkalinity of the serum is more important as it comes into such close contact with the tissues, and may be taken as an index of the changes taking place in the circulating blood. Formerly, litmus was usually employed as an indicator for estimating the alkalinity of the blood, but Moore and Wilson found that sharper readings of the colour changes could be obtained by the use of dimethylamidoazobenzol, and accordingly this was employed in the tests.

The "entire serum" used for tests

Technique.—The blood was collected in Wright's glass capsules, samples being taken from the finger previously cleansed with methylated ether and 5 per cent. formalin. The ends of the capsules were sealed in the ordinary way, the tubes left suspended for twenty-four hours, and then centrifuged so that the serum separated out free from the red cells. If the serum was stained as a result of hæmolysis, it was always discarded.

In each experiment a "control" was used, the blood serum being taken from three healthy persons, whose ages and racial characters corresponded as nearly as possible with those of the patient. In order to avoid the effects of an "alkaline tide," the blood from the patient and from the controls was always secured four hours after the last meal had been taken.

The control

Normal sulphuric acid was employed as the standard acid for titration, and a series of dilutions of the normal acid were made, ranging from a twofold to a sevenfold dilution.

To every 100 c.c. of these acid dilutions were added 8 drops of a 1 per cent. alcoholic solution of dimethylamidoazobenzol, a rich pink colour, sufficiently intense to show the necessary colour changes when mixed with serum, being thereby produced.

¹ Nierenstein, M. (1908), "Observations on the Acidity and Alkalinity of the Blood in Trypanosome Infections." *Annals of Tropical Medicine and Parasitology*, Vol. II., No. 3.

² Moore, B., and Wilson, F. P. (June, 1906), "Clinical Method of Hæmalkalimetry." *Biochemical Journal*, Vol. I.

³ Wright, A. E. (1897), "A Simple Method of Measuring the Alkalinity of the Blood." *Lancet*, Vol. II., p. 719.

The same amount of indicator was always added to the acid solutions, and when ready for use the mixture of acid and indicator was poured into a watch-glass.

Titration of serum against the standard acid solution.—The blood capsules, after centrifuging, were nicked with a file, and the end nearest the serum broken off. The sera of the control individuals was then "pooled" and placed in a separate glass capsule.

A fine capillary pipette, such as is used for estimating the Widal reactions, was inserted into the serum, and a column of serum allowed to run up to a mark made with a blue pencil, about 2 cm. up the tube. The pipette was then withdrawn, a small bubble of air sucked in, and the pipette inserted into the acid until the upper column of the acid was level with the blue mark. In this way equal volumes of serum and acid of a known strength were obtained.

The contents of the capillary tube were then blown out on a clean white slab, thoroughly mixed with the end of the pipette, and aspirated in and out of the capillary tube several times.

The reading
of results

In doing this it was found advisable to avoid the presence of air bubbles in the mixture. If a yellow-coloured fluid resulted, a stronger acid was taken until an orange-red was given, and the dilution of acid which last gave this yellow colour was taken as the index.

If, on the other hand, a pink or orange colour resulted from the first titration, then a weaker acid was used till a yellow colour, free from red, was obtained, and the first dilution of acid which gave this yellow colour constituted the index. As far as possible, all the following estimations were carried out under similar conditions.

All glass tubes, pipettes, and flasks used throughout these experiments were subjected to immersion in pure hydrochloric acid for half an hour, washed in distilled water several times, and then dried in an oven at 130° C., in order to drive off any residual acid. This precaution was taken for the purpose of avoiding any fallacious results due to the presence of alkali in the glass. After this treatment, distilled water left in the tubes for twenty-four hours gave neither an acid nor an alkaline reaction.

Case 1.—An Egyptian soldier, aged 30, with a previous history of irregular fever of two months duration. The general condition of the patient was fair. Several blood examinations failed to show the presence of the parasite of kala-azar. The spleen was enlarged. Splenic puncture showed the presence of Leishman bodies. Soon after diagnosis, and before the patient was placed on any specific line of treatment, the reaction of the blood serum to "dimethyl" was tested, and the result was as follows:—

Pooled normal serum	...	...	0.033 expressed as a fraction of normal
Patient's serum	...	...	0.025 „ „ „

Alkalinity
diminished

This was, comparatively speaking, not a very advanced case, but the alkalinity of the blood of the patient, when compared with the pooled serum, will be seen to be diminished.

Case 2.—An Egyptian soldier, about 28 years of age, with a previous history of recurrent attacks of fever when stationed in the Blue Nile Province. Patient was admitted in a weak condition with a high fever and enlarged spleen. Examination of a peripheral blood film failed to show the presence of malarial or other parasites. The spleen extended to about an inch below the umbilicus, and the lower limit of the liver could be palpated below the costal margin. Splenic puncture showed Leishman bodies to be present.

The serum reaction was tested and showed a diminished alkalinity :—

Pooled normal serum	...	...	0.03	expressed as a fraction of normal
Patient's serum	...	...	0.024	„ „ „

Case 3.—An Egyptian soldier, about 30 years of age. He was admitted into hospital with a previous history of fever in the Kassala Province.

The general condition of the patient was fairly good. Both spleen and liver were enlarged, but the former was not sufficiently enlarged to justify splenic puncture. Repeated examinations of the peripheral blood having given negative results as regards the presence of the malarial parasite, the liver was punctured and a few Leishman bodies were found to be present. The alkalinity of the patient's serum was estimated two days after the diagnosis had been confirmed, and the results were as follows :—

Pooled normal serum	...	...	0.035	expressed as a fraction of normal
Patient's serum	...	...	0.023	„ „ „

There was obviously a marked diminution in the alkalinity of the patient's serum.

Case 4.—A Sudanese boy, aged 14, a native of the Blue Nile Province, admitted to hospital suffering from epistaxis and diarrhoea. The previous history was very indefinite. On admission, a history of epistaxis since childhood was obtained. Patient was somewhat thin and cachectic, and when lying in the prone position an abdominal swelling was apparent. This, on palpation, proved to be an enlarged spleen extending downwards 1 inch below the umbilicus. The liver was also enlarged. Examination of the peripheral blood was negative as regards the presence of malarial parasites, and splenic puncture was resorted to as an aid to diagnosis. Examination of the splenic smears revealed the presence of a large number of Leishman bodies.

The alkalinity of the patient's serum was tested soon after the diagnosis had been confirmed, and was as follows :—

Pooled normal serum	...	...	0.033	expressed as a fraction of normal
Patient's serum	...	...	0.020	„ „ „

A blood count and an estimation of the hæmoglobin were undertaken in this case, and the figures were as follows :—

Red blood corpuscles	...	...	...	...	2,916,000
White blood corpuscles	...	...	...	...	4,680
Hæmoglobin	...	...	...	...	60 per cent.

A differential count showed :—

Polymorphonuclear leucocytes	...	...	...	...	43 per cent.
Large mononuclears	...	...	...	...	32 „
Lymphocytes	...	...	...	...	23 „
Transitionals	...	...	...	...	1 „
Eosinophiles	...	...	...	...	1 „

The patient was treated, soon after admission, with increasing doses of *liquor arsenicalis* for a period of one month, and during this period little change was noted in his general condition.

Clinical
application of
the findings

In view of the fact that a diminution in the alkalinity of the serum was found, and that such clinical signs and symptoms in kala-azar as epistaxis, ulceration of the buccal mucosa, petechiæ, etc., are common features in scurvy, Dr. Balfour suggested that an alkaline treatment might be efficacious.

Accordingly, calcium lactate in 30-grain daily doses was given, and in order at the same time to obtain, if possible, a direct lethal action on the parasite, orsudan in 1-gramme doses was administered intramuscularly four times a week.

The calcium lactate was given in increasing doses until the patient was taking 50 grains *per diem*. The spleen was found to be diminishing in size and the patient's general condition improving slightly. The diarrhoea and epistaxis ceased.

After three weeks' treatment the spleen had retracted to 1 inch above the umbilicus.

The patient's serum alkalinity was again tested and found as follows :—

Pooled normal serum	...	...	0.03	expressed as a fraction of normal
Patient's serum	...	...	0.026	" " "

The alkalinity of the patient's serum had evidently increased after a month's treatment with large doses of calcium lactate, and the general condition of the patient had improved; but judging from the blood count and the hæmoglobin percentage, it was apparent that the prognosis was extremely bad. The count showed as follows :—

Red blood cells	...	...	...	...	2,666,000
White blood cells	...	...	...	...	3,125
Hæmoglobin	...	...	...	...	50 per cent.

A differential count showed :—

Polymorphonuclear leucocytes	...	...	...	...	32 per cent.
Large mononuclears	...	...	...	...	44 "
Lymphocytes	...	...	...	...	21 "
Transitionals	...	...	...	...	2 "
Eosinophiles	...	...	...	...	1 "

In addition to the calcium lactate, hæmâtinics were also administered, but at the end of a fortnight the patient relapsed.

Two days previous to death the hæmoglobin percentage had fallen to 25 per cent.

Post mortem
appearances

The post mortem appearances of the spleen and liver were typical of kala-azar, both organs being enlarged and the liver cirrhotic. Smears revealed numerous Leishman bodies. Ulcers were not present in the intestines. The mucosa of the small intestine was very anæmic and considerably atrophied, and resembled the consistency of thin tissue-paper. This latter condition has been present as a constant pathological condition in almost all the post mortems of these kala-azar cases. With such an atrophic condition of the mucosa of the small intestine the physiological functions of this portion of the gut are practically in abeyance, and consequently the emaciation of the patient progresses steadily in spite of all efforts to combat it.

CONCLUSIONS

It is evident that the alkalinity of the blood in these four cases of undoubted kala-azar was diminished, and it is worth remembering that Rogers, Leishman, Statham, and other observers found that the ideal culture medium for successful cultivation of the Leishman body is an acid one.

If, then, the alkalinity of the blood serum in cases of kala-azar be diminished, it is conceivable that the blood and tissues present a more favourable medium for the Leishman parasite than is usually the case; and, in order to obviate this, it would not seem unreasonable, whatever specific line of treatment be adopted, that drugs should be administered which tend to increase the alkalinity of the blood.

Of the above cases, only one was subjected to an alkaline treatment by calcium lactate, and unfortunately the disease was so far advanced in this instance that the drug had scarcely a fair trial.

A specific drug for the successful treatment of Leishmaniosis has not yet been found. Quinine and various arsenical preparations have not given satisfactory results up to the present,¹ and hence the record of these four cases points to the advisability of employing such remedies as would increase the alkalinity of the blood.

Further, the alkaline reaction obtained in testing the serum of kala-azar cases may be of service in assisting the diagnosis in early cases where splenic enlargement is not marked.

I am indebted to Colonel Mathias, D.S.O., Principal Medical Officer, Egyptian Army, for his kind permission to publish these results, and to the officers of his staff at the Khartoum Military Hospital, for their assistance.

¹ Muir has recently reported favourably on the use of quinine by hypodermic injection, especially in early cases.—*Indian Medical Gazette*, February, 1911.—A.B.



VOLUME A-10

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